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High Production Volume Chemical Challenge Program

Robust Summaries and Test Plan for 1,2-dimethyl-4-nitrobenzene (CAS No. 99-51-4)

Submitted by:

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1.0 Executive Summary

1,2-Dimethyl-4-nitrobenzene (CAS No. 99-51-4), also known as 4-nitro-o-xylene or 4-NOX, is a pale straw yellow colored liquid with an aromatic odor. It is slightly soluble in water and moderately volatile. 1,2-Dimethyl-4-nitrobenzene is generated and used on site as an intermediate in the manufacturing process for pesticides. Potential exposures are thought to be extremely limited, since personal protective equipment is used during sampling events when any dermal contact might occur. Environmental exposures are expected to be extremely rare, since any spills would be handled in an appropriate manner to minimize release to the environment.

The available data for 1,2-dimethyl-4-nitrobenzene (measured, estimated, and from references) were supplemented with data on its isomer, 2,4-dimethyl-1-nitrobenzene (CAS No. 89-87-2), which was used as an analog. Additional supporting information was obtained from data on three nitrotoluene isomers, which differ by a single methyl group from the dimethyl-nitrobenzenes.

In the environment, 1,2-dimethyl-4-nitrobenzene is expected to react with atmospheric hydroxyl radicals. Hydrolysis is not an important fate process. Due to its relatively low octanol-water partition coefficient, 1,2-dimethyl-4-nitrobenzene is not expected to bioaccumulate to a significant degree. Rather, partitioning would be primarily to water and soil compartments. Based on the weight of the evidence about similar compounds (nitro- and methyl-, or amine- and methyl-substituted benzenes), 1,2-dimethyl-4-nitrobenzene is expected to biodegrade, although not readily.

The measured toxicity of 1,2-dimethyl-4-nitrobenzene to aquatic species is low to moderate. These measured data are in close agreement with estimated data and are further supported by data for 2,4-dimethyl-1-nitrobenzene and the nitrotoluenes. The measured toxicity to mammals is low to moderate based upon the oral LD50 for rats (2,636 mg/kg b.w.) and the dermal LD50 for rabbits (> 5,695 mg/kg b.w.). Similar results have been obtained with 2,4-dimethyl-1-nitrobenzene and the nitrotoluenes. *In vitro* tests have yielded both positive and negative results for 1,2-dimethyl-4-nitrobenzene, as well as for the other analogs. There are no *in vivo* genotoxicity data for 1,2-dimethyl-4-nitrobenzene; however, *in vivo* genotoxicity data are available for 2,4-dimethyl-1-nitrobenzene, 2-nitrotoluene and 4-nitrotoluene. Data from a repeated dose study exist for 2,4-dimethyl-1-nitrobenzene, as well as the results of a comparative study on the repeated dose toxicity of the three nitrotoluene isomers. Finally, data on developmental and reproductive toxicity are available for 3-nitrotoluene and 4-nitrotoluene, and 2-year bioassays have been performed with 2-nitrotoluene and 4-nitrotoluene. These data have been used in a read-across manner to fill the health effects information data matrix for 1,2-dimethyl-4-nitrobenzene.

The overall conclusions and recommendations are that adequate information exists for all HPV elements for 1,2-dimethyl-4-nitrobenzene and therefore, no additional testing is required. This information is summarized in Table 1.

Table 1: Summary of Test Plan for 1,2-dimethyl-4-nitrobenzene

SIDS Level I Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-97-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Physicochemical Properties					
Melting point	A	A	A	A	A
Boiling point	A	A	A	A	A
Vapor pressure	A	A	A	A	A
Partition coefficient	A	A	A	A	A
Water Solubility	A	A	A	A	A
Environmental Fate					
Photodegradation	A	A	A	A	A
Hydrolysis	NA	NA	NA	NA	NA
Fugacity	A	A	A	A	A
Biodegradability	R	A	A	A	A
Ecotoxicity					
Acute Fish	A/R	A	A	A	A
Acute Daphnia	A/R	A	A	A	A
Algal Inhibition	A/R		A	A	A
Health Effects					
Acute	A	A	A	A	A
Repeated Dose	R	A	A	A	A
Gene Tox – Mutagenicity	A	A	A	A	A
Gene Tox – Clastogenicity	R	A	A		A
Developmental	R			A	A
Reproductive	R			A	A

A = Adequate Data Exists, R = Read Across, T = Testing Proposed, NA = Not Applicable

2.0 Introduction

2.1 Overview

ARCADIS G&M, Inc., on behalf of BASF Corporation, hereby submits for review and public comment the robust summaries and test plan for 1,2-dimethyl-4-nitrobenzene, also referred to as 4-nitro-o-xylene or 4-NOX (CAS No. 99-51-4), under the United States Environmental Protection Agency's (U.S. EPA) High Production Volume (HPV) Chemical Challenge Program. This document addresses a single HPV sponsored chemical; however, data for its isomer, 2,4-dimethyl-1-nitrobenzene (CAS No. 89-87-2) have been used to support the dataset for 1,2-dimethyl-4-nitrobenzene through a read-across approach. Additional information on the three isomers of nitrotoluene (2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene) is used to strengthen the read-across approach. Data read-across means that physicochemical property and toxicological data from one chemical are used to estimate those same characteristics for another chemical, and is appropriate only when the two chemicals are deemed sufficiently similar in structure that they are likely to have similar chemical and toxicological properties. Typically, the more data-based information there is to support this assumption, the stronger the foundation for the read-across approach will be. The use of structural analogs is consistent with EPA guidance for use of structure-activity relationships (SAR) in the HPV Chemical Challenge Program (EPA, 1999).

The purpose of this plan is to develop physicochemical data, environmental fate and effects data, and mammalian health effects data for 1,2-dimethyl-4-nitrobenzene consistent with the Screening Information Data Set (SIDS). Therefore, this plan summarizes the existing SIDS data for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and the nitrotoluenes, and evaluates the need for testing to fill any data gaps in the SIDS endpoints.

2.2 Methods for Data Review

A review of the scientific literature and BASF Corporation's company data was conducted on the physicochemical properties, environmental fate and effects, and mammalian toxicity endpoints for 1,2-dimethyl-4-nitrobenzene and its isomer, 2,4-dimethyl-1-nitrobenzene, as well as 2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene. Searches were conducted on CAS numbers and chemical names using the following databases: EFDB, ECOTOX, ESIS, and TOXNET (including TOXLINE, MEDLINE, CCRIS, HSDB, DART, GENETOX, and CHEMIDPlus). In addition to these databases, standard handbooks (e.g., CRC Handbook on Chemicals, Hawley's Condensed Chemical Dictionary, Merck Index, etc.) were consulted for physicochemical properties. A variety of individual studies, reports and other data sources were reviewed in development of this test plan. IUCLID profiles were available for 2,4-dimethyl-1-nitrobenzene, 2-nitrotoluene, and 4-nitrotoluene. A test plan and condensed robust summaries for 3-nitrotoluene were available on EPA's HPV web site.

In accordance with U.S. EPA guidance, in those instances where measured physicochemical parameters and environmental fate data were not available, these properties were developed using EPIWIN (EPISuite version 3.12) modeling. EPIWIN is an acronym for the Estimation Programs Interface for Microsoft Windows, and is a package of computer programs developed by the U.S. EPA Office of Pollution Prevention and Toxics (OPPTS) that uses computational methods and structure-activity relationships (SAR) in estimating chemical properties, environmental fate. and aquatic toxicity of organic chemicals. Due to the inherent limitations of SAR approaches, EPIWIN modeling may produce non-realistic estimates; therefore, EPIWIN data are evaluated for reasonableness prior to use.

Lastly, robust summaries were prepared for key studies to provide a detailed summary of the test methods and results. Though several studies may have been evaluated for a particular SIDS endpoint, in general robust summaries were prepared only for the critical study that represented the best available data. Selection of the critical study was based on a review of all studies using the ranking system developed by Klimisch et al (1997), as well as the criteria outlined in the U.S. EPA's methods for determining the adequacy of existing data. Using the IUCLID version 4 format, robust summaries were prepared for 1,2-dimethyl-4-nitrobenzene and for 2,4-dimethyl-1-nitrobenzene, the latter document consisting of an update of the existing IUCLID profile. The existing datasets for the three nitrotoluene isomers are also included in support of this test plan.

3.0 Substance Information for 1,2-dimethyl-4-nitrobenzene

1,2-Dimethyl-4-nitrobenzene (CAS No. 99-51-4) is an aromatic nitrogen compound (Fig. 1). It is also known as 4-nitro-o-xylene. It is a pale straw colored liquid with an aromatic odor. It is slightly soluble in water, but soluble in alcohol and ether. 1,2-Dimethyl-4-nitrobenzene is generated and used on site as an intermediate in the synthesis of pesticides.

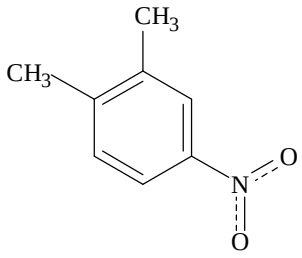


Figure 1. Structure of 1,2-dimethyl-4-nitrobenzene

4.0 Analogs for 1,2-dimethyl-4-nitrobenzene

4.1 EPA Guidance for Use of Analogs

In its SAR guidance for the HPV Chemical Challenge Program, the U.S. EPA states that the most likely analogs for an HPV chemical are those that resemble the candidate chemical in terms of the following:

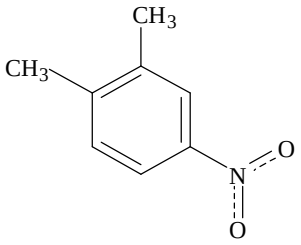
1. molecule structure/size;
2. some substructure that may play a critical functional role;
3. some molecular property (e.g., lipophilicity, electronic and steric parameters); and/or
4. some precursor, metabolite, or breakdown product.

In general, valid analogs should have close structural similarity and the same functional groups as the HPV chemical. In addition, a high correlation is desired between the HPV chemical and the putative analog for the following parameters:

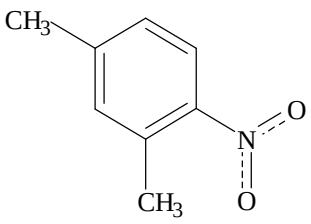
- Ø Physicochemical properties (e.g., physical state, molecular weight, log Kow, water solubility);
- Ø Absorption potential;
- Ø Mechanism of action of biological activity; and
- Ø Metabolic pathways/kinetics of metabolism.

4.2 Structural Similarity and Comparison of Data for 1,2-Dimethyl-4-nitrobenzene and Analogs

The primary selected analog is 2,4-dimethyl-1-nitrobenzene, an isomer of the HPV chemical. Also known as 4-nitro-m-xylene, this compound is believed to possess most of the desired properties for an analog as described in U.S. EPA guidance. The structures of the two chemicals are shown in Figure 2.



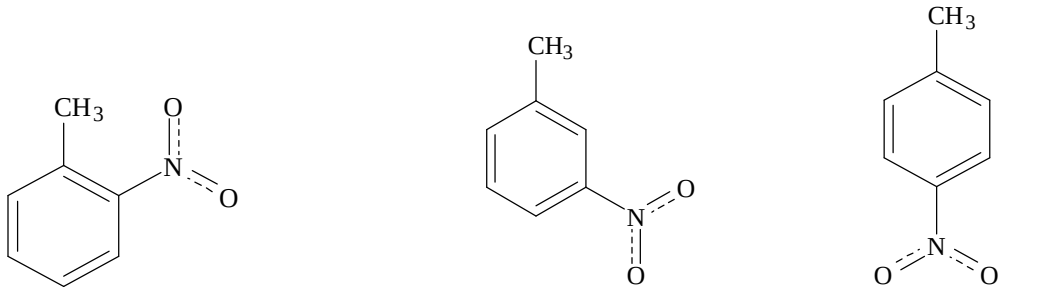
1,2-dimethyl-4-nitrobenzene
(4-nitro-o-xylene)



2,4-dimethyl-1-nitrobenzene
(4-nitro-m-xylene)

Figure 2. Comparison of structures of 1,2-dimethyl-4-nitrobenzene and 2,4-dimethyl-1-nitrobenzene

Additional data were available on a number of SIDS endpoints for the nitrotoluenes, which differ from the nitrobenzenes by having only one rather than two methyl groups. The structures of 2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene are presented in Figure 3. These chemicals are all expected to behave similarly in terms of chemical reactivity.



2-nitrotoluene (o-nitrotoluene)

3-nitrotoluene (m-nitrotoluene)

4-nitrotoluene (p-nitrotoluene)

Figure 3. Structures of 2-nitrotoluene, 3-nitrotoluene and 4-nitrotoluene

Table 2 compares the properties of 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and the nitrotoluenes. The source of the information is classified as “measured”, “reference”, or “estimated.” Measured data were obtained through experimental procedures, while estimated data were obtained through structure-activity correlations. The designation of reference means that the values were obtained from handbooks (such as the Merck Index), from material safety data sheets, or from other sources such as databases. Complete details on the sources of information for each data element are provided in the Robust Summaries submitted with this Test Plan.

Table 2. Comparison of physico-chemical properties of 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and nitrotoluenes

Substance Information and Properties	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (88-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Synonym	4-nitro-o-xylene	4-nitro-m-xylene	1-methyl-2-nitrobenzene	1-methyl-3-nitrobenzene	1-methyl-4-nitrobenzene
Molecular Formula	$C_8H_9NO_2$	$C_8H_9NO_2$	$C_7H_7NO_2$	$C_7H_7NO_2$	$C_7H_7NO_2$
Molecular weight	151.17	151.17	137.14	137.14	137.14
Melting point	30.5 °C (reference)	9 °C (reference)	- 10 °C (reference)[1]	15.5 °C (reference)	51.6 °C (reference)[1]
Boiling point	251 °C (reference)	247 °C (reference)	222 °C (reference)[1]	232 °C (reference)	238.3 °C (reference)[1]
Vapor pressure	0.004786 hPa (reference)	66.5 hPa at 153.7°C (reference)	0.16 hPa at 20ºC (reference)	10 hPa at 89.7ºC (reference)[1]	13 Pa (reference)
Water solubility	100 mg/L (estimated)	133 mg/L (reference)	650 mg/L (reference)[1]	500 mg/L (reference)[1]	442 mg/L (reference)[1]
Partition coefficient (log Kow)	2.91 (reference)[1]	2.91 (estimated)	2.3 (reference)[1]	2.45 (reference)[1]	2.37 (reference)[1]

[1] Database value was reported as being experimentally-derived.

These compounds have very similar physico-chemical properties and would be expected to have very similar environmental fate patterns. The absorption, distribution, metabolism, and elimination of these compounds are also expected to be similar.

Available comparative toxicity data for these compounds support the contention that 2,4-dimethyl-1-nitrobenzene, as well as the nitrotoluenes, are good analogs for 1,2-dimethyl-4-nitrobenzene. As shown in Table 3, ecotoxicity values obtained in studies with fish and daphnia are essentially identical for the two dimethyl-nitrobenzene isomers, and the nitrotoluenes have a similar order of magnitude of aquatic toxicity. Acute oral LD50 values for the rat were similar for all compounds in the group. The nitroaromatic compounds typically cause adverse effects on the blood such as increased methemoglobinemia, anemia, reticulocytosis, and spleen congestion. These compounds can all react with DNA (due to the nitro group), although 2-nitrotoluene is the most potent.

Table 3. Comparison of toxicity of 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and nitrotoluenes

Endpoint[1]	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Acute Fish LC50 (mg/L)	18 (7-day LC50)	10 – 18	7 - 37	25.6 – 32.5	40 - 68
Acute invertebrate LC50 (mg/L)	16	15	5.4 to >77.1	7.5 – 35	4.2 – 11.8
Acute rat oral LD50 (mg/kg)	2,636	2,240 for males; 1,690 for females	891 - 2546	2000 - 2200	2,144 – 4,700 for males; 3,200 for females

[1] All data are measured values. Fish LC50 values are for a 96-h exposure period, except as noted. Invertebrate EC50 values are for a 48-h exposure period.

Based on the weight of evidence it is concluded that 2,4-dimethyl-1-nitrobenzene and the nitrotoluenes are valid analogs for 1,2-dimethyl-4-nitrobenzene and that the environmental fate, ecotoxicity and health effects of these compounds are expected to be very similar. Therefore, data read-across is used for those instances where valid and reliable data are available for 2,4-dimethyl-1-nitrobenzene or the nitrotoluenes but not for 1,2-dimethyl-4-nitrobenzene.

5.0 Data Analysis and Proposed Testing

A summary of the available data for 1,2-dimethyl-4-nitrobenzene and its analogs is shown in Table 1 (in Section 1) and a completed SIDS data matrix is provided in Section 6. These data are discussed below, while details are provided in the Robust Summaries submitted concurrently with this Test Plan.

5.1 Physico-chemical Properties

Values for melting point, boiling point, water solubility, vapor pressure and partition coefficient for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and the nitrotoluenes were obtained from literature references, databases, company Material Safety Data Sheets (MSDS), and estimation (see Table 2). For the needs of the HPV Program, these types of sources provide sufficiently reliable information for many endpoints. The melting point and boiling point for 1,2-dimethyl-4-nitrobenzene were reported in the SRC PhysProp database (accessed through CHEMIDPlus) as 30.5°C and 251°C, respectively. This database also included literature values for vapor pressure (extrapolated to be 0.004786 hPa) and partition coefficient (experimentally determined to be 2.91). The water solubility of 1,2-dimethyl-4-nitrobenzene was estimated to be 100 mg/L using EPIWIN software. These data are considered adequate to fulfill the physico-chemical properties SIDS endpoints.

5.2 Environmental Fate and Pathways

Environmental fate data for 1,2-dimethyl-4-nitrobenzene were developed using EPIWIN model results. The model results indicate that 1,2-dimethyl-1,4-nitrobenzene should behave similarly to 2,4-dimethyl-1-nitrobenzene and the nitrotoluenes. The environmental fate data for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and the nitrotoluenes are summarized in Table 4, below, along with identification of the sources of information (reference, measured, or estimated). Additional information is tabulated in Section 6 and more fully described in the Robust Summaries.

Indirect photodegradation in air was calculated for 1,2-dimethyl-4-nitrobenzene and the analogs using AOPWIN v1.91. The half-life for 1,2-dimethyl-4-nitrobenzene was 10 days, which compares favorably to the half-lives of the analogs. Hydrolysis is not expected to be an important fate process for any of these compounds based upon their structures. Based on the EQC Level III model, it is predicted that 1,2-dimethyl-4-nitrobenzene will be distributed primarily to water (17.6%) and soil (78.7%) under conditions of equal emission to water, soil and air.

No experimental biodegradation data for 1,2-dimethyl-4-nitrobenzene are available, but modeling results (BIOWIN v.402) predict that the timeframe for ultimate biodegradation is weeks to months. 1,2-Dimethyl-4-nitrobenzene belongs to a category of compounds – the nitro- and methyl, or amine- and methyl-substituted benzenes – that are documented in the literature as “intrinsically” biodegradable but not “readily” biodegradable. This means that microbial communities in nature will, given the “right” kind of selective pressure caused by the presence of these compounds at appropriate concentrations (non toxic), acclimate to be able to biodegrade these compounds. Acclimation is a complex process, the end result of which is observed in the laboratory as a new or increased capability by the organism(s) to degrade a compound or a mixture of compounds. The length of the acclimation period can vary a great deal, and usually depends on the source of the inoculum, its history of prior exposure to similar compounds, and the experimental conditions in the laboratory. The available data indicate that biodegradation of 1,2-dimethyl-4-nitrobenzene and its analogs can occur after suitable adaptation. The three nitrotoluene isomers were shown to be biodegraded by activated sludge after an adaptation period (Struijs and Stoltenkamp, 1986). 4-Nitrotoluene was degraded by a *Mycobacterium* sp. (Spiess et al., 1998). A related compound, dinitrotoluene, biodegraded with a half-life of 191 days in soil, but the same compound did not biodegrade using the OECD Test Guideline 301C, which is one of the so-called “ready biodegradation” tests in which there is no opportunity for acclimation of the inoculum (SIAR for SIAM 18, 2004). Based upon the weight of evidence for the analogs, it is expected that 1,2-dimethylbenzene would be biodegradable using an acclimated inoculum.

The environmental fate endpoints for 1,2-dimethyl-4-nitrobenzene are adequately fulfilled by a combination of estimation using acceptable methods and read-across from experimental data on analogs, and no additional testing is proposed.

Table 4. Environmental Fate & Pathways Information for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and nitrotoluenes

Environmental Fate Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Photodegradation half-life	10 days (estimated)	5.5 days (estimated)	13.8 days (estimated)	18.4 days (estimated)	13.8 days (estimated)
Hydrolysis	Expected to be stable to hydrolysis	Expected to be stable to hydrolysis	Expected to be stable to hydrolysis	Expected to be stable to hydrolysis	Expected to be stable to hydrolysis
Fugacity (percent distribution over time assuming equal emissions to air, water and soil)	Air: <3.37%; Water: 17.6%; Soil: 78.7%; Sediment: 0.328% (estimated)	Air: <2.88%; Water: 17.6%; Soil: 79.2%; Sediment: 0.329% (estimated)	Air: 3.49%; Water: 25%; Soil: 71.3%; Sediment: 0.146% (estimated)	Air: 2.98%; Water: 23.2%; Soil: 73.7%; Sediment: 0.174% (estimated)	Air: 2.99%; Water: 24.2%; Soil: 72.7%; Sediment: 0.158% (estimated)
Biodegradation	Does not biodegrade fast (weeks to months) (estimated)	>95% biodegradation after 27 days in Zahn-Wellens test, however air stripping occurred	>90% degradation after 15 days per OECD 302B; >70% degradation after 20 days per OECD 301 D, using adapted sludge	Not readily biodegradable, but inherently biodegradable. 93% degraded after 28 days using adapted sludge	100% degraded after 20 days using adapted sludge per OECD 302B; >90% degraded after 21 days using adapted sludge

5.3 Ecotoxicity

The acute toxicity data are summarized in Table 5 and are more fully described in Section 6 and the Robust Summaries. Acute toxicity tests were conducted with 1,2-dimethyl-4-nitrobenzene using fish, daphnia, and algae, and some chronic toxicity test data are also available (Maas-Diepeveen and Leeuwen, 1986). The results of these tests indicate that 1,2-dimethyl-4-nitrobenzene has low to moderate toxicity to aquatic organisms. For freshwater fish, the 7-day LC50 was 18 mg/L for the early life stage (fertilized eggs/embryos) of *Brachydanio rerio*. The 14-day LC50 for 3-4 week old guppies (*Poecilia reticulata*) was 9.3 mg/L. The 48-h EC50 was 16 mg/L for *Daphnia magna*. For the green alga *Chlorella pyrenoidosa*, the 96-h EC50 was 8.9 mg/L. Although the exposure periods for the fish tests differ from the standard 96 hours, these measured values match quite well with the ECOSAR prediction of 16 mg/L for 96-h LC50 for fish. The measured data also correlate well with the acute toxicity predictions for 1,2-

dimethyl-4-nitrobenzene for daphnids (48-h EC50 of 18 mg/L) and for green algae (96-h EC50 of 12 mg/L). An LC50 value of 5.9 mg/L was also reported for a 21-day exposure with *Daphnia magna* (Maas-Diepeveen and Leeuwen, 1986).

Acute fish and daphnia studies were also conducted for 2,4-dimethyl-1-nitrobenzene. The study with the freshwater fish, *Brachydanio rerio*, was conducted according to GLPs and provided a 96-h LC50 of 10 to 18 mg/L (Hoechst AG, 1987). For *Daphnia magna*, the measured 48-h EC50 was 15 mg/L (Hoechst AG, 1983). There was no algal inhibition study for 2,4-dimethyl-1-nitrobenzene, so the ECOSAR prediction (12 mg/L) is used. Additional ecotoxicity data are available for the nitrotoluene isomers. These include measured values for at least two species of fish for each compound, for *Daphnia magna*, and for green algae (Table 5).

The measured data for 1,2-dimethyl-4-nitrobenzene, although not obtained under GLPs, are judged adequate. The results are in close agreement with estimated data for the compound and comparable to both the measured and estimated data for 2,4-dimethyl-1-nitrobenzene and the three nitrotoluene isomers. Thus, the SIDS ecotoxicity endpoints are adequately covered and no further testing is warranted for 1,2-dimethyl-4-nitrobenzene.

Table 5. Ecotoxicity Information for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene, and nitrotoluenes

Ecotoxicity Endpoint[1]	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Acute fish LC50, 96-h (mg/L)	7-day LC50 = 18 for *Brachydanio rerio* embryos; 16 for fish (estimated)	10 – 18 for *Brachydanio rerio*	7 – 37 for *Oryzias latipes*; 18 – 29 for *Poecilia reticulata* ; 37.1 for *Pimephales promelas*	25.6 – 32.5 for *Pimephales promelas*	40 – 68 for *Cyprinus carpio*; 49 for *Pimephales promelas*; 49 for *Poecilia reticulata*
Acute invertebrate EC50, 48-h (mg/L)	16 for *Daphnia magna*	15 for *Daphnia magna*	5.4 to > 77.1 for *Daphnia magna*	7.5 – 35 for *Daphnia magna*	4.2 – 11.8 for *Daphnia magna*
Algal inhibition EC50, 96-h (mg/L)	8.9 for *Chlorella pyrenoidosa*	12 for green algae (estimated)	48 - 51.7 for *Chlorella pyrenoidosa*	14 for *Chlorella pyrenoidosa*	22.2 for *Chlorella pyrenoidosa*; 25 mg/L for *Scenedesmus obliquus*

[1] Results are for the exposure period specified (96 or 48 h) unless otherwise indicated. All data represent measured values unless otherwise indicated.

5.4 Health Effects

5.4.1 Absorption, Distribution, Metabolism and Excretion

Nitrotoluenes are readily adsorbed via the gastrointestinal tract, the lungs, and to a lesser extent, via the skin (BUA, 1989). For 4-nitrotoluene, distribution is rapid throughout the body and excretion takes place primarily via the urine and only to a minor degree via the feces. The primary metabolic pathway is side-chain or ring oxidation and conjugation with glucuronic acid and inorganic sulfates with subsequent renal excretion. In rats, the involvement of enterohepatic circulation was also observed (SIAR for SIAM 17, 2003).

5.4.2 Acute Toxicity

The results of an acceptable acute oral toxicity test with rats (Fisher, 1975) on 1,2-dimethyl-4-nitrobenzene indicate an LD50 of 2,636 mg/kg b.w. The dermal LD50 for rabbits was greater than 5,695 mg/kg b.w. (Fisher, 1975). A GLP study was performed for 2,4-dimethyl-1-nitrobenzene (Hoechst AG, 1986), indicating that the acute oral LD50 was 2,240 mg/kg b.w for males and 1,690 mg/kg b.w for females. Acute toxicity data for the nitrotoluene isomers are presented in Table 6.

5.4.3 Irritation

The results of acceptable studies indicated that 1,2-dimethyl-4-nitrobenzene was not irritating to the skin or eyes of rabbits (Fisher, 1975). Similarly, the results of GLP studies indicated that 2,4-dimethyl-1-nitrobenzene was not irritating to the skin or eyes of rabbits (Hoechst AG, 1986).

5.4.4 Repeated Dose Toxicity

There are no repeated dose toxicity data available for 1,2-dimethyl-4-nitrobenzene. A feeding study on 2,4-dimethyl-1-nitrobenzene was conducted with male and female rats (TNO-CIVO, 1989) at dose levels of 0, 100, 600, and 3000 ppm. Rats were fed daily for 28 days, followed by a 14-day post-exposure period. The effects observed at the highest dosage included reduced weight gain both during treatment and recovery, as well decreased food consumption, hematological effects, reduced serum protein, increased liver weight and slightly increased pancreas weight. No other dose related effects were seen. The NOAEL was reported as 600 ppm, which can be converted to approximately 43 mg/kg/day (based upon data for 2-nitrotoluene from the Dunnick et al., 1994 study described below, and assuming a feed intake of approximately 16.1 g/day and an average weight of approximately 223.5 g for male rats and a feed intake of approximately 10.4 g/day and and average weight of 143.5 g for female rats).

A comparative 13-week repeated dose study was conducted with 2-nitrotoluene, 3-nitrotoluene, and 4-nitrotoluene (Dunnick et al., 1994; U.S Department of Health and Human Services, 1992). Rats were dosed in feed at 40 to 700 mg/kg/day and mice were dosed in feed at 100 to 1,700 mg/kg/day. There

were no effects on survival, and clinical signs of toxicity were limited to decreases in feed consumption. Decreased body weight gains occurred at the higher dose levels. In rats, histopathologic analyses showed toxicity to kidney, spleen and testis in animals receiving any of the three isomers, and toxicity to the liver and mesothelium in male rats given 2-nitrotoluene. Administration of all three isomers impaired testicular function and increased the estrus cycle in the rat. Liver toxicity was only observed with 2-nitrotoluene in male rats. The only histopathologic evidence for toxicity in mice occurred in the olfactory epithelium in mice receiving 2-nitrotoluene. No liver lesions were noticed in mice, but the three isomers caused increases in relative liver weights. There was no toxicity to the reproductive system in male or female mice treated with any of the three nitrotoluene isomers. The LOAEL in rats was the lowest dose (approximately 42 mg/kg/day in males and 44 mg/kg/day in females) for all three isomers; this was based upon increased relative liver weight for 2-nitrotoluene, non-neoplastic kidney lesions for 3-nitrotoluene, and non-neoplastic lesions of the kidney and spleen for 4-nitrotoluene (Dunnick et al., 1994). The LOAEL in mice for 2-nitrotoluene was approximately 187 mg/kg/day (IUCLID Data Set for 2-nitrotoluene, 2000) based upon degeneration and metaplasia in the olfactory epithelium occurring at the second lowest dose (Dunnick et al., 1994). The LOAEL in mice for 3-nitrotoluene was approximately 101 mg/kg/day based upon increased relative liver weights at all doses in both sexes (American Chemistry Council, Condensed Robust Summary for 3-Nitrotoluene, 2003). The NOAEL in mice for 4-nitrotoluene was 813 mg/kg/day for males and 1075 mg/kg/day for females (SIAR for SIAM 17, 2003), based upon decreased body weight (Dunnick et al., 1994)

5.4.5 Genetic Toxicity

In Ames tests conducted by the U.S. National Toxicology Program using four strains of *S. typhimurium* (TA97, TA98, TA100, and TA1535), with activation, 1,2-dimethyl-4-nitrobenzene and 2,4-dimethyl-1-nitrobenzene were both genotoxic. Both substances were negative in Ames tests using *S. typhimurium* TA98 and TA100 without activation and TA98 with activation (Kawai et al., 1987). An *in vitro* genotoxicity study (OECD 471) was conducted with 2,4-dimethyl-1-ntirobenzene under GLPs (Hoechst AG, 1989). This study employed five strains of *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538), both with and without activation. Positive results were obtained with activation in TA100. In another GLP study, the HGPRT assay (OECD 476) was conducted both with and without activation, demonstrating that 2,4-dimethyl-1-nitrobenzene was negative (CCR, 1992). Other *in vitro* genotoxicity studies showed positive results for *S. typhimurium* and *E. coli*, and negative results for *B. subtilis*. An *in vivo* genotoxicity study (cytogenetic assay with Chinese hamster bone marrow cells), performed according to OECD 475 and under GLPs showed no mutagenicity for 2,4-dimethyl-1-nitrobenzene (CCR, 1991).

2-Nitrotoluene produced positive and negative results with *S. typhimurium* and negative results with *B. subtilis.* This substance was negative in cytogenetic assays with Chinese hamster lung and ovary cells, both positive and negative in sister chromatid exchange assay with Chinese hamster ovary cells, and positive in the unscheduled DNA synthesis test with rat hepatocytes. For 3-nitrotoluene, negative results

occurred in the *S. typhimurium* assay, and in cytogenetics assays with Chinese hamster ovary and lung cells; however, an assay using human lymphocytes was positive. Studies of unscheduled DNA synthesis in whole animals were negative. As discussed in the SIAR for SIAM 17 (2003), using the best quality studies, 4-nitrotoluene was considered non-mutagenic in Ames tests with *S. typhimurium* and *E. coli.* In cultured mammalian cells, 4-nitrotoluene demonstrated the potential to cause mutagenicity in the presence of metabolic activation.

5.4.6 Developmental and Reproductive Toxicity

No information on developmental or reproductive toxicity is available for either 1,2-dimethyl-4-nitrobenzene or 2,4-dimethyl-1-nitrobenzene. However, some information is available for 3-nitrotoluene and extensive information exists for 4-nitrotoluene.

A combined developmental and reproductive toxicity screening study was performed on 3-nitrotoluene in rats. A single dose (300 mg/kg bw/day) was administered by gavage for 90 days prior to mating, and during mating and gestation. Blood and spleen effects typical for nitroaromatic compounds were found in the parental animals, and less severe splenic effects in the offspring. There were no effects indicative of developmental toxicity and no effects on fertility or other reproductive parameters (Ciss, M., 1978; Ciss, M., 1980).

The results of a reproductive/developmental screening test in compliance with OECD TG 421 are available for 4-nitrotoluene (Bayer, 2002). In this study, 12 male and 12 female rats per group received doses of 0, 25, 100, or 400 mg/kg bw/day by gavage. In the high dose group, signs of severe toxicity were observed. Reduced feed intake and body weight gain during lactation were seen in females at 25 and 100 mg/kg bw/day, and this effect was significant at the high dose. Effects on organ weights were recorded in the mid and high dose groups. Insemination index, fertility index, and time to insemination were not affected at any dose. The gestation index was marginally reduced in the high dose group. Live birth index and sex ratio of pups were not affected. Mean pup body weight was significantly reduced at 400 mg/kg bw/day, a dose level at which there was overt maternal toxicity. This was the most sensitive developmental endpoint. The NOAEL for general toxicity was 25 mg/kg bw/day for males; the LOAEL for general toxicity was 25 mg/kg bw/day for females. The NOAEL for reproduction toxicity was also 25 mg/kg bw/day, with the LOAEL at 100 mg/kg bw/day based upon reduced pup birth weight.

Other results for 4-nitrotoluene include a two-generation reproductive study (Aso et al., 2004) in which doses of 0 -160 mg/kg bw/day were orally administered to rats. General toxic effects (lower body weight, increased kidney and liver weights, histopathological signs, clinical signs and deaths were observed; however no abnormalities were seen in the endocrine or reproductive organs or serum hormone levels. In the F1 and F2 offspring, decreases in body weight gain and brain weight were observed at 80 and 160 mg/kg bw/day. The NOAEL was reported as 40 mg/kg bw/day. An extension of this work by Yamasaki et

al., (2005) with extra parameters to detect endocrine disrupting activity did not find any endocrine-mediating influence in parents of offspring.

5.4.7 Carcinogenicity

Two-year bioassays were conducted in rats and mice for both 2-nitrotoluene and 4-nitrotoluene. There was clear evidence of carcinogenic activity in male and female rats and male and female mice for 2-nitrotoluene (U.S. Department of Health and Human Services, 2002). For 4-nitrotoluene, there was equivocal evidence of carcinogenic activity in male rats and mice, some evidence in female rats, and no evidence in female mice (SIAR for SIAM 17, 2003).

5.4.8 Summary of Health Effects Data

The available data for 1,2-dimethyl-4-nitrobenzene fulfill the endpoints for acute toxicity, skin and eye irritation, and *in vitro* genotoxic effects (mutagenicity). There is no information available on the HPV chemical for repeated dose toxicity, clastogenic effects, and developmental or reproductive toxicity. Using the read-across approach, the data from an *in vivo* bone marrow cytogenetic test with 2,4-dimethyl-1-nitrobenzene are adequate to characterize clastogenic effects of 1,2-dimethyl-4-nitrobenzene. Additional supporting data are provided by the results of *in vivo* genotoxicity tests with 2-nitrotoluene and 4-nitrotoluene. A repeated dose toxicity test was conducted with the isomer of the HPV chemical, 2,4-dimethyl-1-nitrobenzene. This is supplemented by a comparative repeated dose study on the three nitrotoluene isomers. Developmental and reproductive toxicity data exist for 3-nitrotoluene and 4-nitrotoluene, including the results of a recent OECD 421 study on the latter compound. In addition, 2-year studies exist for 2-nitrotoluene and 4-nitrotoluene.

The SIDS endpoints relative to health effects for 1,2-dimethyl-4-nitrobenzene and its analogs are summarized in Table 6, with more detailed information provided in Section 6 (SIDS Data Matrix) and in the Robust Summaries submitted with this Test Plan.

Table 6. Health Effects Information for 1,2-dimethyl-4-nitrobenzene, 2,4-dimethyl-1-nitrobenzene and nitrotoluenes

Toxicity Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Acute oral LD50 in rats (mg/kg b.w.)	2,636	2,240 for males; 1,690 for females	Between 891 and 2546	Between 2000 and 2200	2,144 – 4,700 for males 3,200 for females
Mutagenicity	Positive and negative results with *S. typhimurium*	Positive and negative results with *S. typhimurium*; positive with *E. coli*; negative in HGPRT assay; negative with *B. subtilis*; positive in cytogenetic assay	Positive and negative results with *S. typhimurium*; negative with *B. subtilis*; negative in cytogenetic assay; positive and negative in sister chromatid exchange assay	Negative results with *S. typhimurium.* In cytogenetics assay: negative with Chinese hamster ovary and lung cells, positive with human lymphocytes. Negative for unscheduled DNA synthesis.	Based upon best quality studies, considered non-mutagenic in Ames tests with *S. typhimurium* and *E. coli*; in cultured mammalian cells, demonstrated potential to cause mutagenicity in the presence of metabolic activation
Clastogenicity		Negative in Chinese hamster bone marrow test	Positive in unscheduled DNA synthesis with rat and mouse hepatocytes		No increases in micronucleated PCEs in the bone marrow of rats or mice
Repeated dose toxicity		NOAEL = 600 ppm (= approx. 43 mg/kg/day) in a 28-day feeding study with rats	In a 13-week comparative study between nitrotoluene isomers, rats were dosed in feed at 40-700 mg/kg/day and mice dosed in feed at 100-1,700 mg/kg/day. No effects on survival and decreased body weights only seen at higher dose levels. All three isomers showed impaired testicular function and prolonged estrus cycle in rats; all three isomers showed kidney and spleen toxicity in rats; liver toxicity only observed with 2-nitrotoluene and male rats. For rats, the LOAEL was 42 mg/kg/day in males and 44 mg/kg/day in females for all three isomers; this was based upon increased relative liver weight for 2-nitrotoluene, liver lesions for 3-nitrotoluene, and liver and spleen lesions for 4-nitrotoluene. For mice, the LOAEL for 2-nitrotoluene was approx. 187 mg/kg/day based on nasal lesions; the LOAEL for 3-nitrotoluene was approx. 101 mg/kg/day based on increased relative liver weight; and the NOAEL for 4-nitrotoluene was approx. 813 mg/kg/day in males and 1075 mg/kg/day in females based on decreased body weight.		

Toxicity Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-87-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Developmental and reproductive toxicity				Combined reproductive /developmental toxicity screen in rats at 300 mg/kg/day showed no effects indicative of developmental toxicity or effects on fertility or any other reproductive parameters.	Results of OECD 421 repro/developmental toxicity screen in rats: Insemination and fertility indices and time to insemination not affected up to highest dose of 400 mg/kg/day. Gestation index not affected up to 100 mg/kg/day and marginally reduced at 400 mg/kg/day. Mean pup body wt. significantly reduced at 400 mg/kg/day. NOAEL, general toxicity, males: 25 mg/kg/day LOAEL, general toxicity, females: 25 mg/kg/day NOAEL, repro. Toxicity: 25 mg/kg/day LOAEL, repro. toxicity: 100 mg/kg/day Results of two-generation reproductive toxicity study in rats: doses of 0-160 mg/kg/day by gavage; general toxic effects i.e. lower body weight, increased kidney and liver weights, clinical signs and deaths at 80 and 160 mg/kg/day. Decreased body weight gain and brain weight in F1 and F2 offspring; NOAEL = 40 mg/kg bw/day
2-year bioassays in rats and mice			Clear evidence of carcinogenic activity in rats and mice of both sexes		Equivocal evidence of carcinogenic activity in male rats and male mice. Some evidence of carcinogenic activity in female rats, none in female mice

Note: all data are based upon measured values. See robust summaries for details.

5.5 Test Plan Summary

The SIDS Level I endpoints for 1,2-dimethyl-4-nitrobenzene are filled in part by a combination of measured and estimated data for the compound, as listed in Table 7. The adequacy of this information is further supported by comparable data for an isomer of the HPV chemical, 2,4-dimethyl-1-nitrobenzene, and for the related nitrotoluenes. Where certain data are not available for 1,2-dimethyl-4-nitrobenzene, but reliable data exist for the analogs (e.g., biodegradation, repeated dose toxicity, clastogenic effects, developmental and reproductive toxicity), information on the analogs is used in a read-across manner to fill these data gaps. The data on 2,4-dimethyl-1-nitrobenzene and the nitrotoluenes are also used in a read-across manner to further support ecotoxicity data considered adequate for 1,2-dimethyl-4-nitrobenzene. All of the endpoints are adequately filled by a combination of measured, estimated, and read-across data and therefore, no testing is proposed on 1,2-dimethyl-4-nitrobenzene.

Table 7: Summary of Data Gap Analysis for 1,2-dimethyl-4-nitrobenzene

SIDS Level I Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)	2,4-dimethyl-1-nitrobenzene (89-97-2)	2-nitrotoluene (88-72-2)	3-nitrotoluene (99-08-1)	4-nitrotoluene (99-99-0)
Physicochemical Properties					
Melting point	A	A	A	A	A
Boiling point	A	A	A	A	A
Vapor pressure	A	A	A	A	A
Partition coefficient	A	A	A	A	A
Water Solubility	A	A	A	A	A
Environmental Fate					
Photodegradation	A	A	A	A	A
Hydrolysis	NA	NA	NA	NA	NA
Fugacity	A	A	A	A	A
Biodegradability	R	A	A	A	A
Ecotoxicity					
Acute Fish	A/R	A	A	A	A
Acute Daphnia	A/R	A	A	A	A
Algal Inhibition	A/R		A	A	A
Health Effects					
Acute	A	A	A	A	A
Repeated Dose	R	A	A	A	A
Gene Tox – Mutagenicity	A	A	A	A	A
Gene Tox – Clastogenicity	R	A	A		A
Developmental	R			A	A
Reproductive	R			A	A

A = Adequate Data Exists, R = Read Across, T = Testing Proposed, NA = Not Applicable

6.0 SIDS Data Matrix

SIDS Endpoint	1,2-dimethyl-4-nitrobenzene (99-51-4)		2,4-dimethyl-1-nitrobenzene (89-87-2)	
	Value	**Comment**	**Value**	**Comment**
Physicochemical				
Melting point	30.5 °C	SRC PhysProp database	9 °C	MSDS
Boiling point	251 °C	SRC PhysProp database	247.8 °C at 1013 hPa	MSDS
Vapor pressure	0.004786 hPa	Boublik et al., 1984	66.5 hPa at 153.7 °C	MSDS
Partition coefficient (Log Kow)	2.91	Deneer et al., 1987	2.91	EPIWIN
Water Solubility	100 mg/L	WSKOW	133 mg/L at 20 °C	MSDS
Environmental fate				
Photodegradation ($t_{1/2}$)	10 days (12-hr day)	AOPWIN	5.5 days (12-hr day)	AOPWIN
Hydrolysis	Stable		Stable	
Fugacity	3.37% air, 17.6% water, 78.7% soil, 0.328% sediment	EQC Level III	2.88% air, 17.6% water, 79.2% soil, 0.329% sediment	EQC Level III
Biodegradability	Does not biodegrade fast (weeks to months)	BIOWIN	> 95% in 27 day test (Zahn-Wellens); however, losses due to air stripping were noted	Hoechst AG, 1983
Ecotoxicity				
Acute Fish 96-h LC50	18 mg/L for *Brachydanio rerio* embryos (7-day LC50)	Maas-Diepeveen and Leeuwen, 1986	10 – 18 mg/L for *Brachydanio rerio*	Hoechst AG, 1987 (GLP study)
Acute Invertebrate 48-h EC50	16 mg/L for *Daphnia magna*	Maas-Diepeveen and Leeuwen, 1986	15 mg/L for *Daphnia magna*	Hoechst AG, 1983
Algal Inhibition 96-h EC50	8.9 mg/L for *Chlorella pyrenoidosa*	Maas-Diepeveen and Leeuwen, 1986	12 mg/L for green algae	ECOSAR
Toxicity				
Acute Oral LD50	2,636 for rats	Fisher, 1975	2,240 mg/kg (males) and 1,690 mg/kg (females) for rats	Hoechst AG, 1986 (GLP study)
Repeated Dose, NOAEL			600 ppm (approx. 43 mg/kg bw/day) in feed, for rats (28 days)	TNO-CIVO (1989)
Gene Tox – In vitro	Positive and negative in Ames assay	NTP, 1985; Kawai et al., 1987	Positive and negative in Ames assays; Negative in HGPRT assay	Hoechst AG, 1989 (GLP study); NTP, 1985; Kawai et al., 1987; CCR, 1992 (GLP study)
Gene Tox – In vivo			Negative in Chinese hamster bone marrow test	CCR, 1991 (GLP study)
Developmental and Reproductive				

SIDS Endpoint	2-nitrotoluene (88-72-2)		3-nitrotoluene (99-08-1)		4-nitrotoluene (99-99-0)	
	Value	**Comment**	**Value**	**Comment**	**Value**	**Comment**
Physicochemical						
Melting point	-10 ºC	SRC PhysProp database	15.5 ºC	SRC PhysProp database	51.6 ºC	SRC PhysProp database
Boiling point	222 ºC		232 ºC		238.3 ºC	
Vapor pressure	0.16 hPa at 20 ºC	Handbook	10 hPa at 89.7ºC	Handbook value (measured)	13 Pa	SIAR for SIAM 17, 2003
Partition coefficient (log Kow)	2.3	SRC PhysProp database	2.45	SRC PhysProp database	2.37	SRC PhysProp database
Water solubility	650 mg/L at 30º C		500 mg/L at 30º C		442 mg/L at 30 ºC	
Environmental fate						
Photodegradation (t½)	13.8 days	AOPWIN	18.4 days	AOPWIN	13.8 days	AOPWIN
Hydrolysis	Stable		Stable		Stable	
Fugacity	3.49% air, 25% water, 71.3% soil, 0.146% sediment	EQC Level III	2.98% air, 23.2% water, 73.7% soil, 0.174% sediment	EQC Level III	2.99% air, 24.2% water, 72.7% soil, 0.158% sediment	EQC Level III
Biodegradability	>90% degradation after 15 days per OECD 302B; >70% degradation after 20 days per OECD 301 D, using adapted sludge	Hoechst AG, 1993; BUA, 1989	93% degraded after 28 days using adapted sludge	Struijs and Stoltenkamp, 1986	100% degraded after 20 days using adapted sludge per OECD 302B; >90% degraded after 21 days using adapted sludge	Wellens, 1990; Struijs and Stoltenkamp, 1986
Ecotoxicity						
Acute fish 96-h LC50	7 – 37 for *Oryzias latipes*; 18 – 29 for *Poecilia reticulata* ; 37.1 for *Pimephales promelas*	BUA, 1989	25.6 – 32.5 for *Pimephales promelas*	Geiger et al., 1986; Bailey and Spanggord, 1983; Mattson et al., 1976	40 – 68 for *Cyprinus carpio*; 49 for *Pimephales promelas*; 49 for *Poecilia reticulata*	Lang, 1996; Zhao et al., 1997; Yen et al., 2002; Bailey and Spanggord, 1983; Canton et al., 1985
Acute invertebrate 48-h EC50	5.4 to > 77.1 for *Daphnia magna*	BUA, 1989; Maas-Diepeveen and Leeuwen, 1986.	7.5 – 35 for *Daphnia magna*	Maas-Diepeveen and Leeuwen, 1986; Bringmann and Kuhn, 1977	4.2 – 11.8 for *Daphnia magna*	Liu et al., 1977; Zhao et al., 1977; Maas-Diepeveen and Leeuwen, 1986; Canton et al., 1985;
Algal inhibition 96-h EC50	48 - 51.7 for *Chlorella pyrenoidosa*	Maas-Diepeveen, 1986; BUA, 1989	14 for *Chlorella pyrenoidosa*	Maas-Diepeveen, 1986	22.2 for *Chlorella pyrenoidosa*; 25 mg/L for *Scenedesmus obliquus*	Maas-Diepeveen, 1986; Lu et al., 2001

SIDS Endpoint	2-nitrotoluene (88-72-2)		3-nitrotoluene (99-08-1)		4-nitrotoluene (99-99-0)	
	Value	Comment	Value	Comment	Value	Comment
Toxicity						
Acute oral LD50	Between 891 and 2546	Vernot et al., 1977; Hoechst AG, 1975	Between 2000 and 2200	Ciss, M. et al., 1978, 1980	2,144 – 4,700 for males; 3,200 for females	Ciss, M. et al., 1978, 1980; DuPont Chem, 1972.
Repeated dose, NOAEL	In a 13-week comparative study between nitrotoluene isomers, rats were dosed in feed at 40-900 mg/kg/day and mice dosed in feed at 100-2,000 mg/kg/day. No effects on survival and decreased body weights only seen at higher dose levels. All three isomers showed impaired testicular function and prolonged estrus cycle in rats; all three isomers showed kidney and spleen toxicity in rats; liver toxicity only observed with 2-nitrotoluene and male rats. The LOAEL in rats was the lowest dose (approximately 42 mg/kg/day in males and 44 mg/kg/day in females) for all three isomers; this was based upon increased relative liver weight for 2-nitrotoluene, non-neoplastic kidney lesions for 3-nitrotoluene, and non-neoplastic lesions of the kidney and spleen for 4-nitrotoluene (Dunnick et al., 1994; U.S Dept. of Health and Human Services, 2002). The LOAEL in mice for 2-nitrotoluene was approximately 187 mg/kg/day (IUCLID Data Set for 2-nitrotoluene, 2000) based upon degeneration and metaplasia in the olfactory epithelium occurring at the second lowest dose (Dunnick et al., 1994). The LOAEL in mice for 3-nitrotoluene was approximately 101 mg/kg/day based upon increased relative liver weights at all doses in both sexes (American Chemistry Council, Condensed Robust Summary for 3-Nitrotoluene, 2003). The NOAEL in mice for 4-nitrotoluene was 813 mg/kg/day for males and 1075 mg/kg/day for females (SIAR for SIAM 17, 2003), based upon decreased body weight (Dunnick et al., 1994).					
Gene tox – in vitro	Positive and negative results with *S. typhimurium*; negative with *B. subtilis*; negative in cytogenetic assay; positive and negative in sister chromatid exchange assay	BUA, 1989; Kawai, et al., 1987; others (see IUCLID profile)	Negative results with *S. typhimurium*. In cytogenetics assay, negative with Chinese hamster ovary and lung cells, positive with human lymphocytes. Negative for unscheduled DNA synthesis.	Dunnick et al., 1992; Ishidate et al., 1988; Huang et al., 1995; Doolittle, D.J. et al., 1983	Based upon best quality studies, considered non-mutagenic in Ames tests with *S. typhimurium* and *E. coli*; in cultured mammalian cells, demonstrated potential to cause mutagenicity in the presence of metabolic activation	SIAR for SIAM 17, 2003
Gene tox- in vivo	Positive in unscheduled DNA synthesis with rat and mouse hepatocytes	Dunnick et al., 1992			No increases in micronucleated PCEs in the bone marrow of rats or mice	SIAR for SIAM 17, 2003
Developmental and Reproductive			Combined repro/developmental tox screen in rats at 300 mg/kg/day showed no effects indicative of developmental toxicity or effects on fertility or any other reproductive parameters.	Ciss, M. et al., 1978; 1980	Results of combined repro/developmental tox screen (OECD 421) in rats: NOAEL, general toxicity, males: 25 mg/kg/day LOAEL, general toxicity, females: 25 mg/kg/day NOAEL, repro. tox.: 25 mg/kg/day LOAEL, repro. tox.: 100 mg/kg/day	Bayer, 2002

SIDS Endpoint	2-nitrotoluene (88-72-2)		3-nitrotoluene (99-08-1)		4-nitrotoluene (99-99-0)	
	Value	Comment	Value	Comment	Value	Comment
					Results of two-generation repro study in rats: doses of 0-160 mg/kg/day by gavage; general toxic effects i.e. lower body weight, increased kidney and liver weights, clinical signs and deaths at 80 and 160 mg/kg/day. Decreased body weight gain and brain weight in F1 and F2 offspring; NOAEL = 40 mg/kg bw/day	Aso et al., 2004

7.0 References

This list of references is for studies as cited in Sections 1through 5, while a complete list of all data sources reviewed in the development of the Test Plan and Robust Summaries for 1,2-dimethyl-4-nitrobenzene is contained in the Robust Summaries.

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